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STUDIES ON POLLEN STORAGE AND VITALITY DIFFERENCE OF TEA PLANT VARIETIES

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Abstract

Pollen vitality of four different tea plant varieties under different storage conditions were tested by vitro germination method. Results demonstrate that storage temperature and drying processing can influence pollen vitality significantly. Low-temperature dry environment is in favor of pollen vitality maintaining. Pollen samples which are processed by drying agent under room temperature, 4℃ and -20℃ can maintain vitality for 7 days, 20~30 days and 30~40 days, respectively. Pollen sample which haven't processed by dry agent under room temperature, 4℃ and -20℃ can maintain vitality for 1~3 days, 10 days and 20~30 days, respectively. Different tea plant varieties have different pollen storage capacities. Among four testing varieties, Foxiang 3# loses pollen vitality the most quickly, while the Yabukita presents strong pollen storage capacity under all treatments.

Key words: Tea plant varieties; Pollen; Storage; Vitality.

Introduction

Crossbreeding of tea plants has become one of the main ways of resource innovation and breed improvement (Chen *et al*., 2007; Gunasekare *et al*., 2012; Singh *et al*., 2013). Researches on crossbreeding of tea plants are often challenged by asynchronous flowering periods of parents and difficult collection of foreign excellent pollen resources. This requires storage of collected pollens of relevant varieties to prolong service life and maintain vitality of pollens. Main influencing factors of pollen lifetime mainly include genetic factors (variety), temperature and relative humidity (Yang *et al*., 2010; Mesnoua *et al*., 2018; Maryam *et al*., 2017). However, rare researches on the relationship between pollen vitality of different tea plant varieties and storage temperature and humidity were reported (Dafni & Firmage, 2000; Li *et al*., 2008; Murai & Tamotsu, 2000; Novara *et al*., 2017; Tuinstra & Wedel, 2000). In this paper, pollen vitalities of different tea plant varieties under different storage conditions were tested by vitro germination method (Adhikari & Campbell, 1998; Hu *et al*., 2006), aiming to explore a simple and effective storage way of tea plant pollen. Research results provide theoretical supports to innovative utilization of excellent resources, selection of crossbreeding parents, and formulation of effective breeding program.

Materials and Methods

Test materials: Test varieties were Baojing Gold Tea 1#, Yabukita, Fuding white tea and Foxiang 3# from the Test Tea Germplasm Nursery of Hunan Tea Research Institute. The Baojing Gold Tea 1# is a provincial variety of Hunan Province, which belongs to middle-leaf extra-early sprouting variety of shrub (Zhang *et al*., 2012). The Fuding white tea is a national improved variety and belongs to middle-leaf early sprouting variety of *Dungrunga* (Yu *et al*., 2010). The Yabukita is a good variety in Japan and belongs to middle-leaf medium-sprouting variety of shrub (Yamaguchi & Shibamoto, 1981). The Foxiang 3# is the provincial improved variety of Yunnan Province, belongs to large-leaf early sprouting variety of *Dungrunga* (Cai *et al*., 2016).

Test methods

Pollen collection and preparation: Loose milk white buds, which were well developed at middle and upper parts of testing tea plant varieties in the full-bloom stage, were collected on sunny days in middle of October, 2016. These buds were stored in parchment and then put in a dryer with allochroic silicagel for 20-24h. Later, buds were taken by a piece of tweezer at the anthocaulus and beaten gently to make pollen fall on the culture dish. Collected pollen samples were packed by parchment, about 1g for each.

Firstly, a dried clean10ml finger-type test tube was filled with 1/3 tube of allochroic silicagel which was fixed with few degreasing cotton. Secondly, pollen samples were put into the finger-type test tube and then fixed with few degreasing cotton. Finally, the finger-type test tube was covered and sealed by preservative film.

Storage conditions and method design: Pollen storage temperatures in this experiment were set room temperature (The room temperature test was conducted from October 10th~25th 2015, with the mean daily minimum/maximum temperature of 17℃/26℃, and the mean daily temperature of 22.7℃.), 4℃ and -20℃. Each storage temperature had two test groups: with or without drying. Therefore, a total of six test groups were prepared. Pollen vitality under room temperature was tested at 0d, 3d, 5d, 7d and 9d, while pollen vitality under 4℃ and -20℃ were tested at 0d, 10d, 20d, 30d, 40d, 50d and 60d, respectively.

Pollen vitality test: Pollen vitality was tested by vitro germination method (Adhikari & Campbell, 1998; Hu *et al.*, 2006). In other words, vitality of pollen samples was judged according to germination rate at different time and under different storage conditions after separated from tea plants. Culture medium was prepared by 1% agar + 300mg/L $Ca(NO_3)_2$ + 200mg/L $MgSO_4$ + 100mg/L KNO_3 + 10% sugar + 100mg/L H_3BO_3 (Brewbaker & Kwack, 1963). In the pollen vitality test, pollen samples were scattered on the glass slide with culture medium uniformly by the dissecting needle. The glass slide was put in the culture dish with wet filter paper and cultured in 25℃ constant-temperature incubation for 6h. Pollen germination rate was observed. Each group had three parallel tests and three views were determined for each glass slide. Pollen germination was defined as the pollen tube length larger than the pollen diameter (Maryam & Jaskani, 2017). Germination rate (number of germinated pollen / total pollen number ×100%) at each observation view was recorded and the mean result was taken as the pollen germination rate of this group.

Data analysis: Data were processed by SPSS 20.0 and measurement data were expressed by mean ± standard deviation ($\bar{x} \pm s$). Variance analysis of repeated measurement data was used to compare pollen germination rates of different varieties at different time. $P<0.05$ represents statistical significance of difference.

Results and analysis

Pollen vitality of different tea plant varieties under room temperature: Fresh pollen germination rates of Baojing Gold Tea 1#, Yabukita, Fuding white tea and Foxiang 3# are higher than 90%, valuing 94.22%, 93.66%, 94.40% and 90.38%, respectively. It can be seen from Fig. 1 that pollen germination rate of all varieties under room temperature decreases as time goes on. At the 7d of dry storage under room temperature, pollen germination rates of all varieties are still higher than 50%. The Baojing Gold Tea 1# has the highest pollen germination rate (63.12%). When no drying agent is used, pollen germination rates of all varieties at 3d drop dramatically. The pollen germination rates of Fuding white tea and Foxiang 3# are only 18.02% and 12.06%, basically inactivated. The pollen germination rates of Yabukita and Baojing Gold Tea 1# maintain at 77.81% and 55.75%. At 5 d, pollen germination rates of four varieties decrease to 0%. A paired T-test has been made for desiccant and non-desiccant treatments, and got a P-value much less than 0.01 (p-value=1.85e-12), which indicated a significant difference between desiccant/non-desiccant treatment groups.

According to variance analysis of repeat measurement data under room temperature and dry conditions, with respect to main effect of time factor, $F=125.42$ and difference has statistical significance ($p<0.01$), indicating that pollen germination rate changes with time. Based on pairwise comparison, there are significant differences except pollen germination rates at 3d and 5d ($p>0.05$). For main effect of variety factor, $F=6.00$ and difference has statistical significance ($p<0.01$), indicating that different varieties have different pollen germination rates. The pairwise comparison discloses significant difference only between the Baojing Gold Tea 1# and Foxiang 3#. This reflects that among four test varieties, the Baojing Gold Tea 1# has significantly higher pollen vitality than Foxiang 3%. The interactive effect of time and variety shows no statistical significance ($p>0.05$), implying that effect of time factor is independent from variety factor.

Pollen vitality of different tea plant varieties under 4℃: Under 4℃ storage conditions, pollen germination rates of four varieties decrease with the increase of storage time. The group with drying agent has lower decreasing rate compared to the group without drying agent. Figure 2 reveals that Baojing Gold Tea 1# and Yabukita could be stored for 30d under 4℃, when their pollen germination rates are 62.17% and 56.13%, respectively. The Fuding white tea has 60.15% pollen germination rate at 20d and loses pollen vitality at 30d. The Foxiang 3# lose pollen vitality at 20d, showing a pollen germination rate of only 48.66%. When no drying agent is used, pollen germination rates of all four varieties decline sharply at 10d. The Foxiang 3# lose pollen vitality and the pollen germination rate is only 35.47%. Baojing Gold Tea 1# and Yabukita could be stored for 20d, with pollen germination rates of 58.69% and 56.54%. Particularly, the pollen germination rates of Yabukita at 30d and 40d still maintain 47.70% and 30.95%, while pollen germination rates of rest varieties are 0%. The Fuding white tea could be stored for 10d and the pollen germination rate at 20d is only 30.21%.

According to variance analysis of repeat measurement data under 4℃ and dry conditions, with respect to main effect of time factor, $F=227.25$ and difference has statistical significance ($p<0.01$), indicating that pollen germination rate changes with time. Based on pairwise comparison, there are significant differences despite pollen germination rates at 20d and 30d as well as pollen germination rates at 40d and 50d ($p>0.05$). For main effect of variety factor, $F=29.30$ and difference has statistical significance ($p<0.01$), indicating that different varieties have different pollen germination rates. Based on the pairwise comparison, no statistically significant difference was found between the Fuding white tea and Yabukita as well as between Fuding white tea and Foxiang 3# ($p>0.05$), but rest pairwise comparisons had significant differences. The interactive effect of time and variety has statistical significance ($p<0.05$), implying that effect of time factor changes with variety.

Pollen vitalities of different tea plant varieties under -20℃: Under -20℃ storage conditions, pollen germination rates of four varieties decrease with the increase of storage time. The group with drying agent has lower decreasing rate compared to the group without drying agent (Fig. 3). When drying agent is used, pollen germination rates of the Baojing Gold Tea 1# and Yabukita at 10d and 20d decrease slightly. Their pollen germination rates at 40d are still higher than 50%. The Fuding white tea and Foxiang 3# could be stored for 30d. Among four varieties, the Foxiang 3# shows the quickest reduction in pollen germination rate, decreasing to 58.15% at 20d. When no drying agent is used, the pollen vitality of Yabukita could be maintained for 40d. The pollen germination rates at 50d and 60d are still as high as 40.38% and 36.00%. Pollen vitality of the Baojing Gold Tea 1# and Fuding white tea could be maintained for 30d, with higher than 50% pollen germination rates. The Foxiang 3# loses pollen vitality at 20d and the pollen germination rate at 30d is 0%.

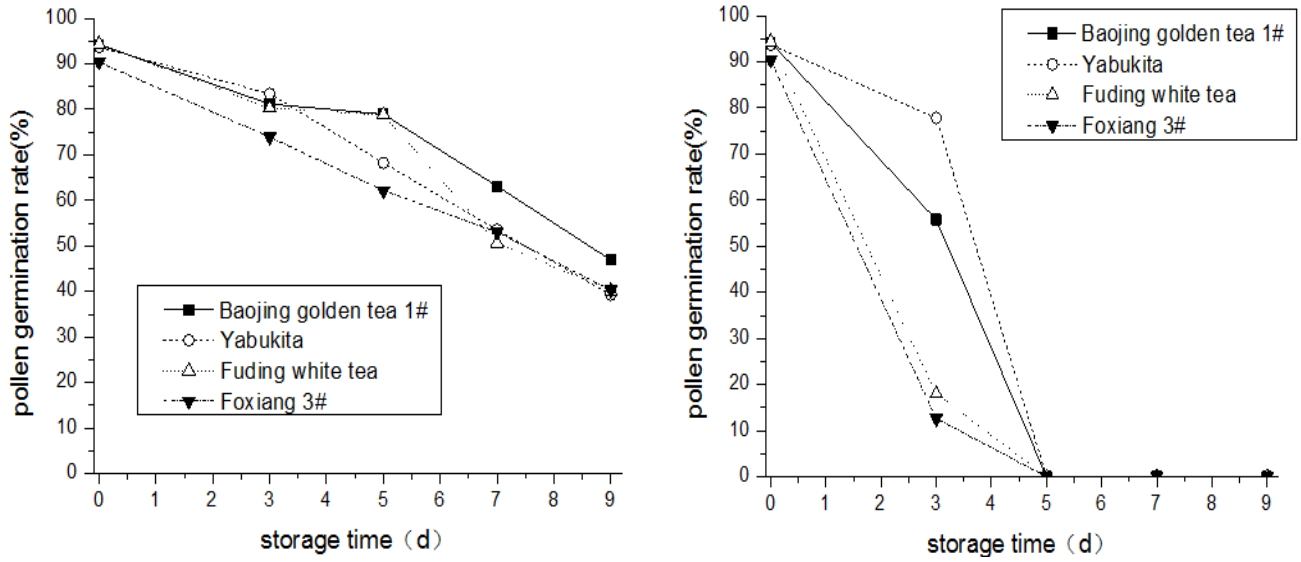

Fig. 1. Pollen germination rates of different tea plant varieties under room temperature (%) (Left: Desiccant treated; Right: Non Desiccant).

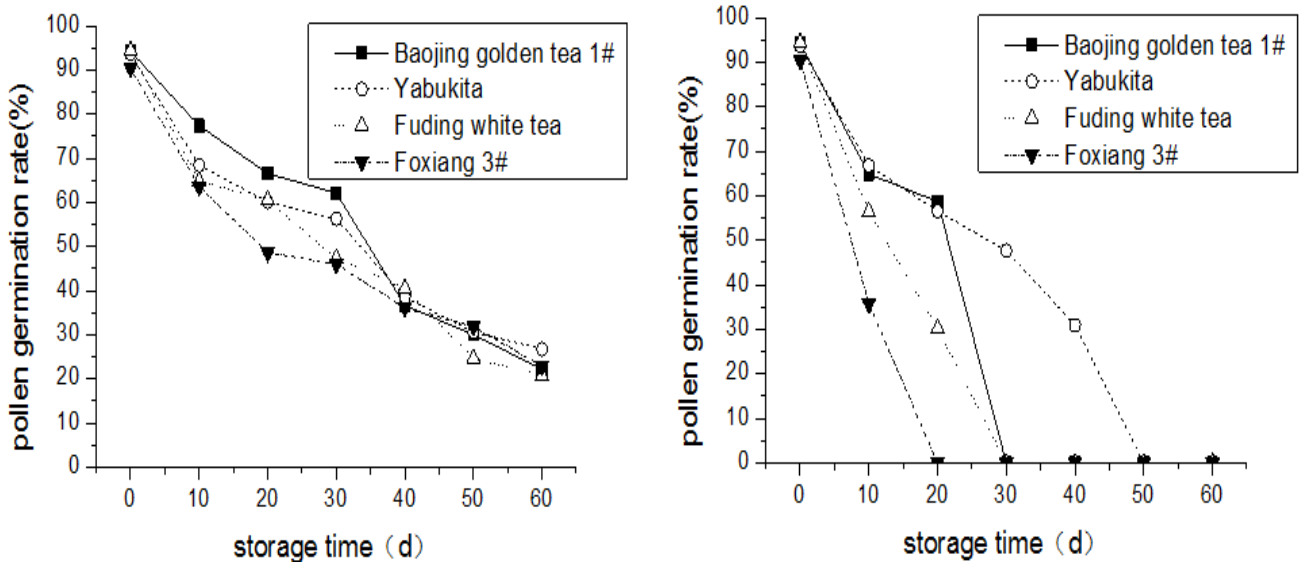

Fig. 2. Pollen germination rates of different tea plant varieties under 4℃ (%) (Left: Desiccant treated; Right: Non Desiccant).

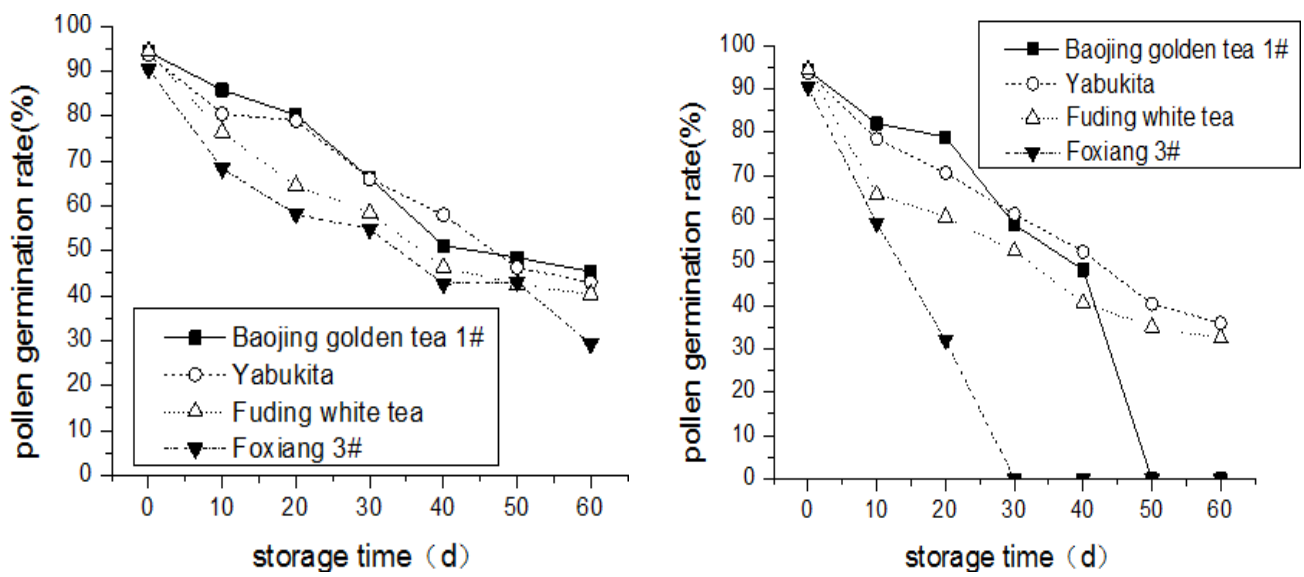

Fig. 3. Pollen germination rates of different tea plant varieties under -20℃ (%) (Left: Desiccant treated; Right: Non Desiccant).

According to variance analysis of repeat measurement data under -20℃ and dry conditions, with respect to main effect of time factor, F=182.83 and difference has statistical significance ($p<0.01$), indicating that pollen germination rate changes with time. Based on pairwise comparison, there are significant differences despite pollen germination rates at 40d and 50d as well as pollen germination rates at 50d and 60d ($p>0.05$). For main effect of variety factor, F=35.33 and difference has statistical significance ($p<0.01$), indicating that different varieties have different pollen germination rates. Based on the pairwise comparison, no statistically significant difference is found between the Baojing Gold Tea 1# and Yabukita ($p>0.05$), but there are significant differences in rest pairwise comparisons. The interactive effect of time and variety has statistical significance ($p<0.05$), implying that effect of time factor changes with variety.

Discussions

The cross pollination between tea plants are often challenged by asynchronous flowering periods of parents and difficult collection of foreign excellent pollen resources, which requires the storage of collected pollens of relevant varieties to prolong service life and maintain vitality of pollens. Hence, this work set three temperature conditions including room temperature, 4℃ and -20℃, according to actual storage and transportation conditions. A series of temperatures (especially -20℃) is just set for the pollen collected from tea plant, with the aim of Prolonging their vitality, which have nothing to do with the growing conditions of tea plant.

Since the loss of pollen vitality is a continuous process without a distinct boundary, it has to determine a threshold to express test results when studying pollen vitality (Borghezan *et al*., 2011; Karipidis & Douma, 2011). In this paper, the pollen survival time of Kumar *et al*., (1995) was used as the measurement standard: the pollen germination rate higher than 50% was defined as the pollen survival time.

(1) Storage temperature influences pollen survival time significantly. With and without drying agent. Pollen germination rates under three temperatures are -20℃>4℃> room temperature. For groups with drying agent, pollen vitality of all varieties can be stored for 7d under room temperature. Pollen vitality of all varieties can be stored for 20d under 4℃, while pollen vitalities of the Baojing Gold Tea 1# and the Yabukita can even be maintained for 30d. Under -20℃, pollen vitality of all varieties can be maintained for 30d, while pollen vitalities of the Baojing Gold Tea 1# and the Yabukita can even be maintained for 40d. For groups without drying agent, the Baojing Gold Tea 1# and the Yabukita can be stored for 3d under room temperature, whereas the Fuding white tea and Foxiang 3# can be stored for 1-2d; the Baojing Gold Tea 1# and the Yabukita can be stored for 20d, the Fuding white tea can be stored for 10d, and the Foxiang 3# can be stored for only 7d. Under -20℃, pollen survival time of the Foxiang 3# is only 10d, but pollen vitality of rest three varieties can last for 30d and the pollen vitality of Yabukita can even last for 40d.

(2) The use of drying agent influences pollen survival time significantly. Under different storage temperatures, groups with drying agents have longer pollen survival time compared to groups without drying agents. Under room temperature, four varieties with drying agents can be stored for 7d, while those without drying agents can only be stored for 1-3d. Under 4℃, the Foxiang 3# with drying agent loses pollen vitality at 20d, and the Baojing Gold Tea 1# and the Yabukita with drying agent still have 62.17% and 56.31% pollen germination rates at 30d. However, the Baojing Gold Tea 1# and the Yabukita without drying agent maintain pollen vitality for only 20d, the Fuding white tea can be stored for 10d and the Foxiang 3# can only be stored for less than 10d. Under -20℃, the Baojing Gold Tea 1# and the Yabukita with drying agent can maintain pollen vitality for 40d, while the Fuding white tea and Foxiang 3# can maintain pollen vitality for 30d. Nevertheless, the Foxiang 3# without drying agent can maintain pollen vitality for only 10d, while rest three varieties can maintain pollen vitality for 30d.

(3) Pollen storage time is co-related with tea plant varieties. According to variance analysis of repeat measurement data, differences of the variety factor under all three temperatures have statistical significance, indicating that different varieties have different pollen germination rates. According to pairwise comparison, the Baojing Gold Tea 1# has significantly longer pollen survival time than the Foxiang 3# under room temperature. Under 4℃ and -20℃, statistically significant differences are recognized in all pairwise comparisons except for those between Fuding white tea and Yabukita, between Fuding white tea and Foxiang 3#, between Baojing Gold Tea 1# and Yabukita. Among four tea plant varieties, the Foxiang 3# loses pollen vitality the most quickly, while Yabukita present strong storage capability all the time. According to interactive effect of time and variety, the time effect on pollen germination rate is independent from the variety factor under room temperature, but varies under rest two test temperatures.

Attentions should be paid to that the Foxiang 3# is large-leaf variety, and others are middle-leaf variety. Whether the poor pollen storage of Foxiang 3# is related with pollen particle size needs further researches.

Conclusions

Asynchronous flowering periods of parents and difficult collection of foreign excellent pollen resources for distance crossbreeding are common problems in crossbreeding of tea plant varieties. Pollen storage technology is the key solution to these problems and also has important significance to germplasm resources storage and utilization. According to experimental results in this paper, (1) all test varieties have strong fresh pollen vitality and have pollen germination rates higher than 90%, indicating that the pollen collection by putting buds in the dryer for 20-24h is an efficient and reliable method. (2) Collected fresh pollens may be used on that day or the second day. If they have to be stored for a short time, collected pollens be kept in flask or culture dish and then put in the dryer. This storage method is effective in 7d

under room temperature. It is a common, effective, simple and necessary pollen storage method in tea plant crossbreeding. (3) Upon asynchronous flowering periods of parents, the early flowering pollen samples be collected firstly and wrapped by parchment before stored in a sealed container with drying agent. According to storage time, they shall be kept under 4℃ (20-30d) or -20℃ (30-40d). (4) When introducing excellent pollen resources from other places, the best storage and transportation method is to wrap pollens with parchment and then put them in a closed container (e.g. finger-type test tube) with drying agent (e.g. allochroic silicagel). They can be stored under room temperature (7d) or 4℃ (20-30d).

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References

Adhikari, K.N. and C.G. Campbell. 1998. In vitro germination and viability of buckwheat (*Fagopyrum esculentum* Moench) pollen. *Euphytica*, 102(1): 87-92.

Borghezan, M., A.D. Clauman, D.A. Steinmacher, M.P. Guerra and A.I. Orth. 2011. *In vitro* viability and preservation of pollen grain of kiwi (*Actinidia chinensis* var. *deliciosa* (a. chev.) a. chev). *Crop Breed. & Appl. Biotechnol.*, 11(4): 338-344.

Brewbaker, J.L. and H.K. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *Amer. J. Bot*., 50(9): 859-865.

Cai, L.W., Y.H. Zhou, S.X. Yang, Y.L. Zhang, B. Hu, H.J. Guo and S.B. Wu. 2016. A study on the adaptability of Yunnan tea cultivars in southern Fujian. *Asian Agri. Res.*, 08(10): 93-96.

Chen, L., Z.X. Zhou and Y.J. Yang. 2007. Genetic improvement and breeding of tea plant (*Camellia sinensis*) in China: from individual selection to hybridization and molecular breeding. *Euphytica*, 154(1-2): 239-248.

Dafni, A. and D. Firmage. 2000. Pollen viability and longevity: Practical, ecological and evolutionary implications. *Plant Syst. & Evol.*, 222(1-4): 113-132.

Gunasekare, M.T.K., M.A.B. Ranatunga, J.H.N. Piyasundara and J.D. Kottawaarchchi. 2012. Tea genetic resources in Sri Lanka: collection, conservation and appraisal. *Int. J. Tea Sci.*, 8(3): 51-60.

Hu, D.Y., Z.S. Ling, J.X. Ting and W.U. Jun. 2006. Characteristics of pollen germination and pollen tube growth of prunus mume *In vitro*. *Acta Botanica Boreali-Occidentalia Sinica*, 26: 1846-1852.

Karipidis, C. and D. Douma. 2011. Tomato pollen storage at freeze and cryogenic temperature-effects on viability and fecundity. *Acta Horticulturae*, 908: 257-263.

Kumar, A., R.K. Chowdhury and O.S. Dahiya. 1995. Pollen viability and stigma receptivity in relation to meteorological parameters in pearl millet. *Seed Sci. Technol.*, 23: 147-156.

Li, J., J. Chen, Z.H. Zhang and Y.H. Pan. 2008. Proteome analysis of tea pollen (*Camellia sinensis*) under different storage conditions. *J. Agri. & Food Chem.*, 56(16): 7535-7544.

Maryam, M.J. Jaskani and S.A. Naqvi. 2017. Storage and viability assessment of date palm pollen. *Methods Mol. Biol.*, 1638: 3-13.

Mesnoua, M., M. Roumani and A. Salem. 2018. The effect of pollen storage temperatures on pollen viability, fruit set aXnd fruit quality of six date palm cultivars. *Scientia Horticulturae*, 236: 279-283.

Murai and Tamotsu. 2000. Effect of temperature on development and reproduction of the onion thrips, thrips tabaci lindeman (Thysanoptera: Thripidae), on pollen and honey solution. *Appl. Entomol. & Zool.*, 35(4): 499-504.

Novara, C., L. Ascari, V. La Morgia, L. Reale, A. Genre and C. Siniscalco. 2017. Viability and germinability in long term storage of corylus avellana pollen. *Scientia Horticulturae*, 214: 295-303.

Singh, S., R.K. Sud, A. Gulati, R. Joshi, A.K. Yadav and R.K. Sharma. 2013. Germplasm appraisal of western himalayan tea: A breeding strategy for yield and quality improvement. *Genetic Resour. & Crop Evol.*, 60(4): 1501-1513.

Tuinstra, M.R. and J. Wedel. 2000. Estimation of pollen viability in grain sorghum. *Crop Sci.*, 40(4): 968.

Yamaguchi, K. and T. Shibamoto, 1981. Volatile constituents of green tea, gyokuro (*Camellia sinensis* L. var. Yabukita). *J. of Agri. & Food Chem.*, 29(2): 366-370.

Yang, S.M., W.X. Song, Y.C. Tang, L. Ma, Y.G. Wang and H. Cheng. 2010. Studies on pollen viability of section thea and hybrids of species. *Chinese Agri. Sci. Bull.*, 26(8): 115-118.

Yu, J. Z., H.T. Huang, M.Z. Yao and Y.J. Yang. 2010. Analysis of genetic diversity and relationship of half-sib tea cultivars related to fuding dabai and yunnan daye using est-ssr markers. *J. Tea Sci.*, 30(3): 184-190.

Zhang, X.S., J.G. Peng, C.X. Long, Y. Yang, X.C. Yang, Y. Zhao, T.S. Xiang, Z. Liu, J.Z. Shi, J. Ning and G.B. Yu. 2012. The breeding of early budding, high amino acid content and high quality new green-tea cultivar Baojing Huangjincha 1. *J. Tea Comm.*, 39(3): 11-16.

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